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Antiplasmodial activity of a series of 1,3,5-triazine-substituted polyamines

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Polyamine biosynthesis and function has been shown to be a good drug target in some parasitic protozoa and it is proposed that the pathway might also represent a target in the malaria parasite *Plasmodium falciparum*. A series of 1,3,5-triazine-substituted polyamine analogues were tested for activity against *Plasmodium falciparum in vitro*. The series showed activity against the parasites and were generally more active against the chloroquine-resistant line K1 than the chloroquine-susceptible line NF54. Simple unbranched analogues had better activity than analogues carrying branched or cyclic central chains. Addition of multiple triazine units in general led to increased activity of the compounds.

Keywords: *Plasmodium falciparum*, malaria, parasites, protozoa, polyamine metabolism

Introduction

Malaria remains one of the principal diseases of the developing world with an estimated 500 million cases each year and around two million deaths.[1] In the absence of effective vaccines, chemotherapy represents the mainstay of malaria control. Resistance to currently used antimalarials, particularly chloroquine and the antifolates, is seriously impeding efforts to control the disease.[2] New drugs are urgently needed in the fight against malaria. Polyamine metabolism has been shown to be a good target for chemotherapeutic intervention in diseases caused by other protozoa such as human African trypanosomiasis,[3] where the ornithine decarboxylase inhibitor eflornithine has been registered for use against late-stage disease.[4]

Polyamines are essential for cell proliferation and differentiation. Interference with their biosynthesis or function can block cellular growth and the polyamine pathway has been investigated with regard to anticancer therapy.[5] Malaria parasites have a great propensity for rapid proliferation, and interference with polyamine function in these cells is likely to be detrimental. Eflornithine, used in conjunction with bis(benzyl)polyamine analogues, cured rodent malaria models,[6] although used alone eflornithine has no curative affect in human malaria. An *S*-adenosylmethionine decarboxylase inhibitor, methylglyoxalbis(guanyl) hydrazone (MGBG), blocked growth of the erythrocytic stages of *Plasmodium falciparum in vitro*.[7] Moderate antiplasmodial activity was also seen with a series of *N*-alkylated putrescine derivatives,[8] and dicyclohexylamine, an inhibitor of spermidine biosynthesis, also showed antiplasmodial activity.[9]

We previously reported a series of 1,3,5-triazine-substituted polyamine analogues, which were developed specifically to interfere with polyamine metabolism in African trypanosomes.[10] Several of the compounds showed marked trypanocidal activity. We report here the evaluation of the activity of this series against *P. falciparum in vitro*.

Materials and methods

The syntheses of the compounds has been reported previously.[10] *In vitro* activity against erythrocytic stages of *P. falciparum* was determined using a [^{3}H]hypoxanthine incorporation assay.[11,12] One strain susceptible to known antimalarial drugs (*P. falciparum* NF54) and another resistant to chloroquine and pyrimethamine (*P. falciparum* K1) were used in the assays, and all test compounds were compared for activity with the standard drugs chloroquine (Sigma C6628) and artemisinin (arteannuin, qinghaosu; Sigma 36,159-3). Compounds were dissolved in dimethyl sulfoxide at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL) + Albumax (5 g/L) and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial doubling dilutions of each drug were prepared in 96-well microtitre plates and incubated in a humidifying atmosphere at 37°C; 4% CO_2, 3% O_2, 93% N_2.

After 48 h, 50 μL of [^{3}H]hypoxanthine (0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate cell

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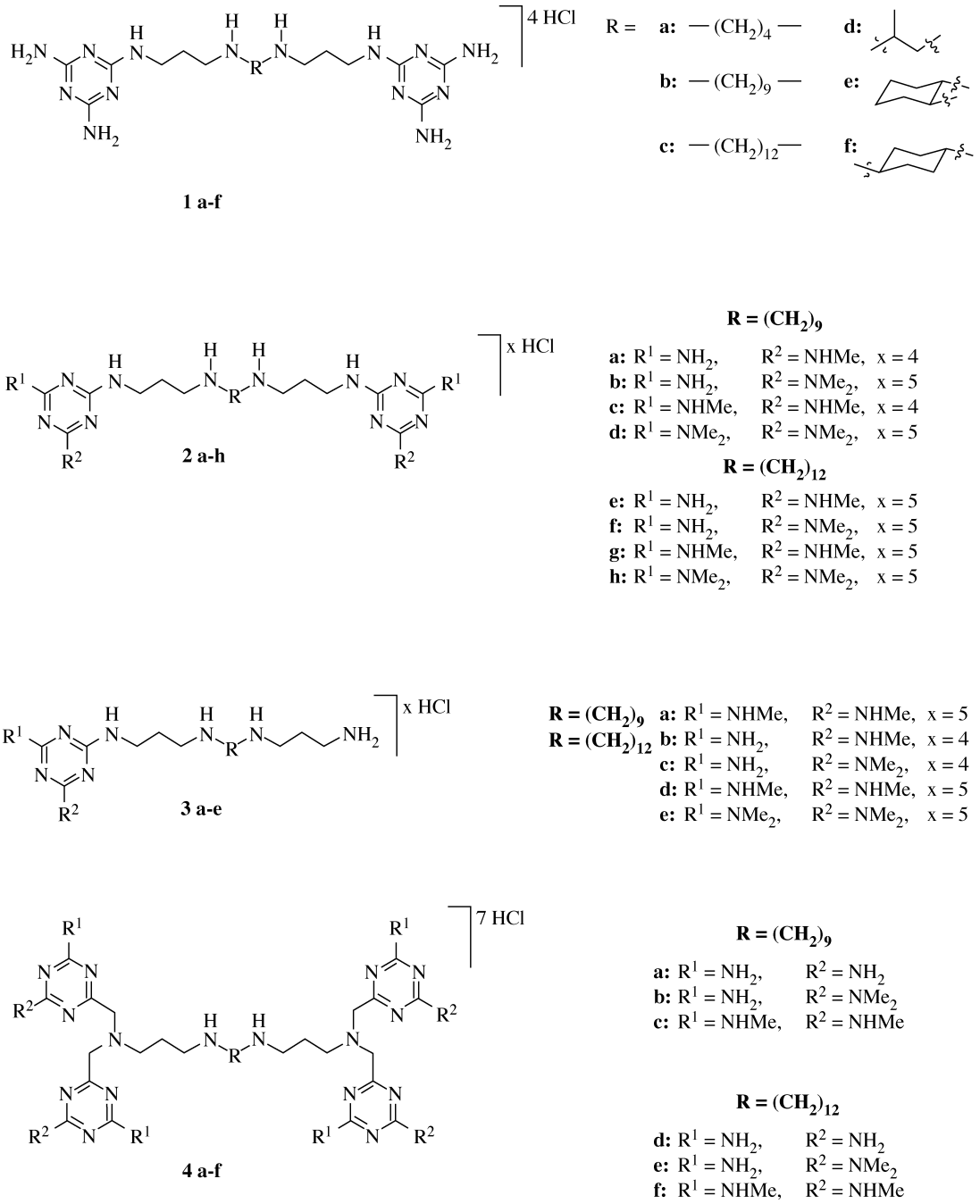

Figure 1. Structures of the 1,3,5-triazine-substituted polyamines used in this study.

harvester (Wallac, Zurich, Switzerland), and the red blood cells were transferred onto a glass fibre filter and then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate liquid scintillation counter (Wallac). IC_{50} values were calculated from sigmoidal inhibition curves using Microsoft Excel.

Results

The structures of the compounds are shown in Figure 1. Compounds were tested against two strains of *P. falciparum*. The strains used were NF54, which is susceptible to known antimalarial drugs, and K1, which is resistant to chloroquine and pyrimethamine (Table 1). Compounds were generally more active against the resistant K1 strain than against the susceptible NF54 strain. For simple substituted derivatives, compound 1, activity increased as the length of the central chain increased in the order butyl < nonyl < dodecyl. Branched chain and cyclic central chains had little activity. No major effect was noted on increasing the methylation of the triazine ring for compounds 2a–d and 2e–h. However, against NF54 strain, there was a small increase in activity on methylation, whilst for the K1 strain, there is a small decrease in activity on methylation. No major effect was seen on having one triazine unit compared with two. In general the polyamines substituted with one triazine appeared less active than those substituted with two triazines (comparing 2e–h with 3b–e). The

Table 1. Antimalarial activity of compounds against NF54 and K1 strains of *P. falciparum*

	NF54	K1	L6 rat myoblast cells
Compound	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
1a	>17.6	>8.80	353
1b	4.383	0.155	ND
1c	0.618	0.142	295
1d	>18.1	>18.1	362
1e	>16.9	>16.9	338
1f	>16.9	>16.9	338
2a	3.95	0.216	285
2b	1.93	0.410	274
2c	1.49	0.291	289
2d	0.579	0.745	37.7
2e	0.809	0.229	ND
2f	0.149	0.244	0.9
2g	0.470	0.372	143
2h	0.173	0.335	6.8
3a	0.582	0.180	135.2
3b[a]	2.033	0.408	127
3c[b]	0.618	0.743	39.9
3d	1.724	0.391	57.7
3e	0.831	0.450	14.2
4a	>7.5	>7.5	220
4b	0.164	0.135	177
4c	0.072	0.154	177
4d	1.563	0.747	188
4e	0.0698	0.0622	105
4f	0.0477	0.0519	76.6

ND, not determined.
[a]3b contains ~10% of bis-adduct 2e.
[b]3c contains ~15% of bis-adduct 2f.
The IC_{50} values are the means of four values of two independent assays carried out in duplicate. They were determined by linear interpolation between the two adjacent drug concentrations above and below the 50% incorporation line.

most effective compounds were tetra-substituted methylene-bridged compounds 4, especially where they were methylated. These compounds also showed best activity against *Trypanosoma brucei*.[10] *In vitro* data did not point to cytotoxicity; however, acute toxicity, manifest in muscular tremor, motor-function disorder and distress that necessitated immediate killing of the animal was observed when compounds 2e, 4b, 4c or 4e were applied to mice at concentrations >10 mg/kg, precluding further *in vivo* evaluation. Thus in this case, the cellular assay was not a good indicator of toxicity towards the animal. The toxicity may be caused by an effect on a specific signally event or receptor, rather than general cytotoxicity.

Discussion

In this report we show that triazine-substituted polyamine analogues have antiplasmodial activity against erythrocytic stages of the parasite *in vitro*. It was previously shown[6] that bis(benzyl) polyamine analogues had activity against malaria parasites in the 0.2–14 μM range, and we demonstrate here that these triazine-substituted compounds are also active in this range. *N*-alkyl putrescine derivatives have also been shown to be weakly active against *Plasmodium*, although benzyl derivates were shown to give improved activity in this case as well. Interestingly, chloroquine/pyrimethamine-resistant parasites are more susceptible to these analogues than are susceptible parasites; however, we have not made mechanistic studies that can be used to help interpret this observation. In another study evaluating the effects of spermidine biosynthesis inhibition,[9] data were presented showing a 3-fold higher spermidine content in wild-type NF54 parasites compared with a chloroquine-resistant strain. If polyamine deficiency were a common phenotype of chloroquine-resistant lines this could explain the enhanced susceptibility of these cells to polyamine analogues, which might act by displacing polyamines from their normal roles in cells.

However, it is currently not clear how these triazine-substituted polyamine analogues actually exert their action. In rodents, *Plasmodium yoelii*-infected erythrocytes have been shown to have enhanced polyamine uptake.[13] The parasites may induce specific transporters for these metabolites and *Plasmodium knowlesi*-infected erythrocytes have been shown to have an up-regulated putrescine-specific uptake system.[14] It is also possible that the triazine-substituted analogues are preferentially taken into *Plasmodium*-infected erythrocytes via the new permeation pathway that is induced at the plasma membrane of infected erythrocytes.[15] It will be of interest to determine how these analogues enter infected erythrocytes and exert a toxic effect on the parasites. We cannot rule out the possibility that the compounds are oxidized by serum components prior to exerting a toxic effect. Interestingly, it has recently been shown that pentamidine and other diamidine compounds are active against malaria parasites grown *in vitro*,[16] with efficacy in the submicromolar range. It has been proposed that diamidines exert their plasmocidal effect by binding to ferriprotoporphyrin IX and preventing the polymerization of haem that accumulates to toxic levels in these parasites. It is feasible that the triazine-substituted polyamine analogues could act in a similar fashion. Further work is required to elucidate modes of action of these compounds.[13]

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